REVIEW ARTICLE

CPE Toward Individualized Cholesterol-Lowering Treatment in End-Stage Renal Disease

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There is broad evidence that lowering low-density lipoprotein (LDL) cholesterol will reduce cardiovascular risk. However, in patients on maintenance hemodialysis treatment, lowering LDL cholesterol is not as effective in preventing cardiovascular complications as in the general population. Cholesterol is either endogenously synthesized or absorbed from the intestine. It has been suggested that the benefit of using statins to prevent atherosclerotic complications is less pronounced in people with high absorption of cholesterol. Recent data indicate that patients on hemodialysis have high absorption of cholesterol. Therefore, these patients may benefit from dietary counseling to reduce cholesterol intake, from functional foods containing plant sterols and stanols, and from drugs that interfere with intestinal absorption of sterols (i.e., ezetimibe, bile acid resins, and sevelamer). This review discusses cholesterol homeostasis and the perspective of personalized treatment of hypercholesterolemia in hemodialysis.

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Introduction

HYPERCHOLESTEROLEMIA IS AMONG the main risk factors for atherosclerotic vascular disease.[1,2] However, in patients on maintenance hemodialysis treatment, the relationship between circulating cholesterol and vascular endpoints appears to be less consistent.[3] A large-scale Japanese study has indeed reported multivariate-adjusted, positive correlations of non-high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels with incident myocardial infarction.[4] However, an unadjusted inverse relationship between total cholesterol and all-cause death was reported in the same cohort.[4] Inverse associations of total cholesterol levels with mortality have also been observed in the CHOICE study.[5] The inverse relationships of total cholesterol with all-cause death and the less pronounced positive associations of non-HDL and LDL cholesterol levels with vascular endpoints are most likely caused by malnutrition, protein-energy wasting, and frailty, which are prevalent in end-stage renal disease.[4–6] Nevertheless, LDL in patients on chronic hemodialysis is considered harmful because of the accumulation of particularly atherogenic LDL subfractions.[7]

There is broad evidence that lowering LDL cholesterol will reduce cardiovascular mortality in the general population.[8–10] However, the effectiveness of statin treatment to prevent cardiovascular complications is less evident in hemodialysis.[11,12] In the 1,255 participants of the 4D study, 20 mg of atorvastatin did not reduce the primary endpoint compared with placebo.[11] Moreover, 10 mg of rosuvastatin had no significant effects on cardiovascular events in the 2,776 participants of the AURORA trial.[12]

The work presented here aims to discuss cholesterol homeostasis in chronic dialysis patients and potential implications of cholesterol homeostasis for lipid-lowering treatment. Moreover, different strategies of cholesterol-lowering treatment with a focus on therapies targeting intestinal cholesterol absorption are reviewed.

Cholesterol Homeostasis

Circulating cholesterol is derived from diet or from endogenous de novo synthesis.[13] Intestinal absorption of cholesterol is a multistep process.[14] Cholesterol and plant sterols are taken up into enterocytes after hydrolysis by lipases in mixed micelles together with bile acids and other lipids (Fig. 1).[14] The Niemann Pick C1-like 1 (NPC1L1) protein is crucial in the process of internalization of micelles into the enterocyte brush border (Fig. 1).[14] The ATP

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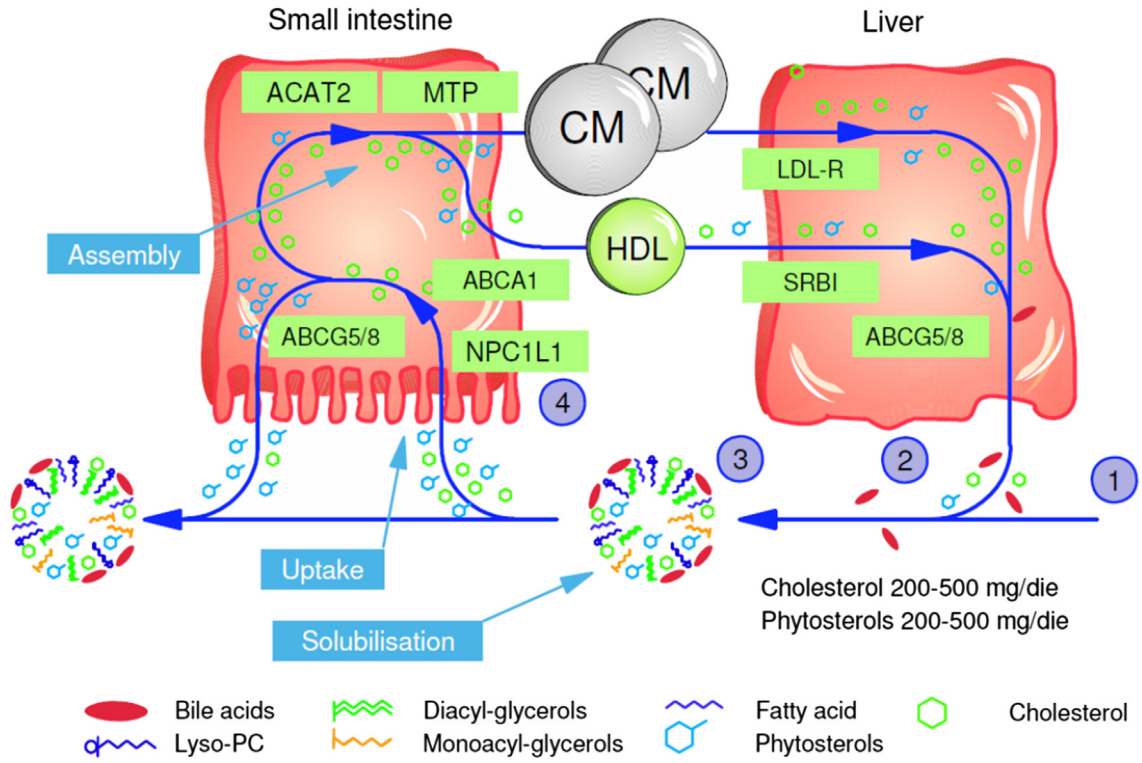

Figure 1. Cholesterol homeostasis with a focus on the intestine and the liver with contact points of different LDL cholesterol-lowering strategies that affect the absorption of sterols. 1. Reduced dietary intake of cholesterol. 2. Reduction of cholesterol content of mixed micelles due to the use of plant sterols. 3. Reduced solubilization of cholesterol in mixed micelles by bile acid resins and sevelamer. 4. Inhibition of NPC1L1 mediated cholesterol uptake by ezetimibe. ABCA1, ATP binding cassette transporter A1; ABCG5/8, ATP binding cassette co-transporters G5 and G8; ACAT2, acetylcoenzyme-A cholesterol acyl transferase 2; CM, chylomicron; HDL, high-density lipoprotein; LDL-R, low-density lipoprotein receptor; MTP, microsomal triglyceride transfer protein; NPC1L1, Niemann Pick C1-like 1 protein; SRBI, scavenger receptor class B type 1.

binding cassette co-transporters G5 and G8 (ABCG5 and ABCG8) re-secrete cholesterol and plant sterols, that have not been esterified by acetyl-coenzyme-A cholesterol acyl transferase 2, back from the enterocyte into the intestinal lumen (Fig. 1).[14] Because most of cholesterol is esterified, whereas plant sterols are poorly esterified, re-secretion is much higher for plant sterols than for cholesterol. Hence, approximately 50% of intestinal cholesterol, but only 1% to 2% of plant sterols, are finally incorporated into chylomicrons or HDL and leave the enterocyte at the basolateral membrane via lymph (Fig. 1).[14] Incorporation of cholesterol into lipoproteins is mediated by the microsomal triglyceride transfer protein and the ATP binding cassette transporter A1 (Fig. 1).[14] The LDL receptor and scavenger receptor class B type 1 are involved in the uptake of cholesterol and plant sterols from chylomicrons and HDL, respectively, into the liver (Fig. 1).[14]

Recent genome-wide association studies have shown variants in the *ATP binding cassette transporter G8* (ABCG8) and *ABO* genes to consistently correlate with intestinal cholesterol absorption and consecutive changes in cholesterol synthesis.[15] In addition to genetic regulation, cholesterol absorption and synthesis are influenced by metabolic factors.[13,16,17] For example, obesity, type 2 diabetes, and fatty liver predispose to high synthesis and low absorption of cholesterol.[16-18] On the other hand, type 1 diabetes and frailty are associated with low synthesis and high absorption of cholesterol.[17,19] Age appears to be inversely related to cholesterol absorption and synthesis.[16,17] Taken together, the balance of cholesterol absorption and synthesis is individually regulated. In general, cholesterol absorption and synthesis are interrelated so that people with high cholesterol absorption display low cholesterol synthesis and vice versa.[16,17] Using stable isotope methods, circulating noncholesterol sterols and cholestanol have been demonstrated to indicate cholesterol absorption and synthesis.[20,21] The association with cholesterol homeostasis measured with isotope methods was even stronger for the ratios of the noncholesterol sterols and cholestanol to cholesterol than for the uncorrected values.[20,21] Several methods, which are based on gas chromatography, gas-liquid chromatography, and/or mass spectrometry, have been developed to analyze these compounds.[17,22] Circulating campesterol and sitosterol, representing the 2 most abundant plant sterols, can be used to estimate

cholesterol absorption.[13,20] The disadvantage of measuring circulating plant sterols is that they also reflect the fruit and vegetable content of the diet.[13,20] This may confound associations of cholesterol homeostasis with vascular disease.[23] Circulating cholestanol, which is structurally highly similar to cholesterol, can be used instead of plant sterols to estimate cholesterol absorption.[13,21,23] In contrast to circulating plant sterols, cholestanol does not increase in response to high intake of plant-derived foods.[23] Circulating lathosterol, which is a cholesterol precursor, is most commonly used to estimate cholesterol synthesis.[13,20] Such as cholesterol, noncholesterol sterols and cholestanol are transported in the blood stream as components of lipoproteins.[24] Most noncholesterol sterols and cholestanol in the circulation are carried in LDL particles. However, they can also be found in other lipoprotein subfractions, with cholestanol and plant sterols being abundant in HDL and lathosterol being abundant in very-low-density lipoprotein.[24]

Efficacy of Lipid-Lowering Strategies Depends on Cholesterol Metabolism

Hypercholesterolemia may be due to increased cholesterol absorption, increased cholesterol synthesis, or both.[13] On the basis of this pathophysiology, there are 2 main concepts to treat hypercholesterolemia. Firstly, cholesterol synthesis may be reduced with statins.[8-10] Secondly, cholesterol absorption may be reduced with a diet low in cholesterol,[25,26] with plant sterols or stanol-enriched functional foods,[27] or with drugs interfering with cholesterol absorption.[14,28,29] There is some evidence that the individual balance of cholesterol absorption and synthesis has an effect on the response to lipid-lowering treatment.[30-32] For example, inhibition of cholesterol synthesis with statins appears to be less effective in people with high cholesterol absorption.[30,33] In contrast, inhibition of cholesterol absorption with ezetimibe, plant sterols, or stanols appears to be less efficient in people with low cholesterol absorption.[31,32] Dietary interventions and the use of other cholesterol absorption inhibitors such as bile acid resins and sevelamer should also be more effective in high cholesterol absorbers compared with high cholesterol synthesizers.

Drugs Interfering With Intestinal Cholesterol Handling

Ezetimibe

By binding to NPC1L1, ezetimibe inhibits the vesicular internalization of this molecule, which is a crucial step in intestinal cholesterol absorption.[14,34] Reduced cholesterol absorption in patients treated with ezetimibe is partly compensated for by an increase of cholesterol synthesis.[35] Nevertheless, 10 mg ezetimibe is associated with a decrease in LDL cholesterol of approximately 15% when used as monotherapy or in combination with a statin.[34,36,37] In addition to LDL cholesterol, circulating plant sterols are significantly reduced by treatment with ezetimibe.[38] Therefore, ezetimibe is also used to treat patients with sitosterolemia.[39] The question whether inhibition of sterol absorption with ezetimibe treatment will reduce cardiovascular risk in the general population has so far not been convincingly answered. However, the ongoing IMPROVE-IT trial is being performed to answer the latter question.[40] The results of this study are expected to be published in 2014.[32]

Bile Acid Resins

Bile acids, mainly cholic acid and chenodeoxycholic acid, are synthesized in the liver and are excreted via bile.[34,41] Approximately 90% of bile salts are reabsorbed in the ileum and colon.[34,41] Bile acid resins bind bile salts in the intestine, resulting in decreased reabsorption of bile acids.[34,41] In consequence, there is a need for increased production of bile acids from cholesterol. This leads to decreased intracellular cholesterol in the liver and increased uptake of LDL cholesterol by the liver, thereby reducing LDL cholesterol concentrations.[34,41] Cholestyramine and colestipol are the 2 traditional bile acid resins used for lowering cholesterol.[41] Cholestyramine is slightly more potent than colestipol, with 10 g per day reducing LDL cholesterol as monotherapy or in combination with statins by approximately 15%.[41] Colesevelam is a novel bile acid resin that shows markedly increased potency compared with the 2 aforementioned bile acid resins. Of relevance, bile acid resins have been demonstrated to prevent progression of atherosclerosis[42] and to prevent cardiovascular events and cardiovascular mortality in a randomized controlled study.[43] However, today cholestyramine and colestipol are hardly used anymore for lowering cholesterol because their intake frequently causes gastrointestinal side effects (e.g., nausea, gastrointestinal discomfort).[41] However, the novel bile acid resin colesevelam appears to have fewer side effects.[41]

Sevelamer

Sevelamer is a phosphate-lowering resin that has been developed from colesevelam; therefore, it does not contain calcium.[44] Probably because of its bile acid binding property,[44] the use of sevelamer is associated with a decrease of LDL cholesterol of approximately 30%.[45] In a subgroup analysis in European participants in the TTG-Study, sevelamer was associated with less progression of aortic and coronary calcification compared with calcium-containing agents.[46] In agreement, sevelamer was associated with less progression of vascular calcification compared with traditional phosphate-lowering agents in the RIND study.[47] Sevelamer treatment was also associated with reduced all-cause mortality in the RIND study.[48] In contrast, the DCOR study did not show overall survival benefit of treatment with sevelamer.[49] Cardiovascular mortality remained

unchanged by sevelamer in the RIND and DCOR studies.[48,49]

Combinations of Cholesterol-Lowering Agents Affecting Intestinal Cholesterol Metabolism

The addition of ezetimibe to a bile-acid-resin-based regimen reduced LDL cholesterol by another 19%.[50] Moreover, the addition of plant sterols in patients receiving ezetimibe resulted in a further reduction of LDL cholesterol of approximately 7%.[38] The effects of combination therapies of sevelamer with plant sterols, ezetemibe, or bile acid resins on lipids have, to our knowledge, not been tested so far in randomized controlled trials.

Cholesterol Homeostasis in End-Stage Renal Disease

Two recent studies have investigated cholesterol homeostasis in patients on maintenance hemodialysis treatment.[51,52] Rogacev and colleagues found that 113 people on hemodialysis had higher cholesterol absorption and lower cholesterol synthesis compared with 220 controls.[51] Fukushima and colleagues similarly reported increased cholesterol absorption in 94 hemodialysis patients compared with 58 controls.[52] Rogacev and colleagues also made the interesting observation that hemodialysis patients with increased cholesterol absorption may have increased mortality compared with those with low cholesterol absorption.[51] Although both studies were small and need replication in larger cohorts, they indicate that patients on chronic hemodialysis may profit from inhibition of cholesterol absorption.

Dietary Strategies to Reduce Circulating Cholesterol in Chronic Hemodialysis

There is broad evidence that high dietary cholesterol intake is associated with increased circulating cholesterol and higher risk for cardiovascular death.[53] Moreover, dietary interventions that include lowering cholesterol intake have been shown to reduce LDL cholesterol[25,26] and to prevent cardiovascular complications.[54] It is likely that particularly people with high cholesterol absorption may be vulnerable to a diet rich in cholesterol. This may apply to people on chronic hemodialysis.[51,52] In agreement, prehemodialysis dietitian care was associated with lower circulating cholesterol and reduced mortality during the first year of dialysis in a cohort of 156,440 patients.[55] Khoueiry and colleagues performed a study using food frequency questionnaires to estimate the dietary habits of people on chronic hemodialysis in a U.S. outpatient dialysis unit.[56] They found that most people did not follow many of the dietary recommendations of the American Heart Association for reducing the risk of cardiovascular disease.[56] However, cholesterol intake was surprisingly low, which may already indicate good compliance of the patients with dietary advice to reduce cholesterol intake.[56] Approaches to further reduce LDL cholesterol in hemodialysis patients will thus have to go beyond dietary counseling.

Plant Sterols and Stanols for Lipid Lowering in Hemodialysis Patients

Dietary intake of plant sterols has been known to reduce LDL cholesterol since the 1950s.[57] In the 1990s, the use of plant sterols and stanols became popular because they could then be incorporated into various foods such as spreads and dairy foods.[58] The regular intake of foods containing approximately 2 g of plant sterols or stanols is associated with a decrease in LDL cholesterol of approximately 10% to 13%.[27,28] Therefore, the use of plant sterols and stanols has been recommended by the American Heart Association.[59] Moreover, the European Society of Cardiology and the European Atherosclerosis Society have suggested that foods enriched with plant sterols may be considered for individuals with elevated total and LDL cholesterol values in whom the total cardiovascular risk assessment does not justify the use of a cholesterol-lowering drug.[60] Because they interfere with cholesterol absorption, plant sterols and stanols may be considered helpful in the treatment of hypercholesterolemia in chronic hemodialysis patients. Despite the marked decrease of LDL cholesterol, there is still a debate on whether the use of plant sterols and stanols is safe.[58] These concerns are primarily based on the association of sitosterolemia with an early onset of cardiovascular disease.[39] Sitosterolemia is a very rare genetic disorder caused by mutations in the ABCG5 and ABCG8 genes.[39] It is characterized by tendon and tissue xanthomas and approximately 50-fold elevated serum plant sterol levels.[39] In contrast to sitosterolemic patients, frequent users of plant-sterol-enriched foods only have modestly increased circulating plant sterols.[61] A recent meta-analysis has shown that moderately elevated circulating plant sterols are not associated with increased cardiovascular risk.[62] The associations of common variants in the ABCG8 and *ABO* genes with cardiovascular disease[63-65] most probably are not mediated by elevated plant sterol levels, either.[15] Rather, high cholesterol absorption causes an increase in cardiovascular risk.[15] Large-scale clinical studies testing for associations between circulating plant sterols on cardiovascular risk are currently not available in patients with end-stage renal disease. These data may help to further elucidate whether the use of plant sterols and stanols is safe in these patients.

Interpretation of the 4D, AURORA, and SHARP Trial Results With a Focus on Cholesterol Homeostasis

Statins did not significantly reduce major cardiovascular events in the 4D and AURORA trials.[11,12] According to Rogacev and colleagues and Fukushima and colleagues,

hemodialysis is associated with high absorption of sterols.[51,52] These observations may partly explain why treatment with statins was not as successful as expected in end-stage renal disease.[11,12] On the other hand, these observations suggest that patients on chronic hemodialysis may potentially profit from cholesterol absorption inhibitors. The SHARP study has investigated the effect of ezetimibe plus simvastatin treatment on major cardiovascular events compared with placebo in chronic kidney disease.[66] In the entire cohort of 9,270 participants, the combination therapy significantly reduced atherosclerotic events during a median follow-up of 4.9 years.[66] Although no such benefit could be observed in the subgroup of 3,023 patients on hemodialysis, the study supports beneficial effects of ezetimibe treatment on atherogenesis in renal disease.[66] An alternative explanation why the combination of simvastatin and ezetimibe was effective in reducing cardiovascular risk in the SHARP study could be the fact that ezetimibe reduces the intestinal absorption not only of cholesterol and plant sterols but also of atherogenic oxidized sterols.[67,68]

Conclusions

Summing up, there is some evidence that inhibition of cholesterol absorption may be beneficial to cardiovascular health in hemodialysis patients.[32,33,51,52] Keeping a healthy diet will decrease LDL cholesterol, but the achievable reductions may be not strong enough with this single approach. The SHARP study showed that treatment with the cholesterol absorption inhibitor ezetimibe plus simvastatin prevents major cardiovascular events in patients with chronic kidney disease who were partly on hemodialysis.[66] However, SHARP did not prove that particularly ezetimibe will prevent major vascular events.[66] Indeed, there is some evidence that treatment with sevelamer is beneficial to cardiovascular health in patients on hemodialysis.[46–48] The efficacy of plant-sterol- and stanol-enriched functional foods and bile acid resins to prevent atherosclerosis has not been tested in the setting of chronic hemodialysis.

Perspectives

1) Future studies should address the scientific question whether cholesterol absorption will predict the effectiveness of statin treatment to reduce cardiovascular risk in hemodialysis patients. Our hypothesis is that hemodialysis patients with relatively low absorption of cholesterol might profit from statin treatment. However, we expect those with relatively high absorption to be mainly nonresponders.
2) It remains to be shown whether an association of circulating plant sterols with vascular disease exists in hemodialysis patients. If there is no association, then this may argue against the atherogenic effects of plant sterol supplementation in hemodialysis patients.

Practical Application

Patients on chronic hemodialysis appear to have high cholesterol absorption. Therefore, dietary counseling and drugs that inhibit intestinal cholesterol absorption may be instrumental to reduce cardiovascular risk in these patients.

References

1. Emerging Risk Factors CollaborationDi Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*. 2009;302:1993-2000.
2. Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. *N Engl J Med*. 2008;358:1240-1249.
3. Krane V, Winkler K, Drechsler C, Lilienthal J, März W, Wanner C, German Diabetes and Dialysis Study Investigators. Association of LDL cholesterol and inflammation with cardiovascular events and mortality in hemodialysis patients with type 2 diabetes mellitus. *Am J Kidney Dis*. 2009;54:902-911.
4. Shoji T, Masakane I, Watanabe Y, Iseki K, Tsubakihara Y, Committee of Renal Data Registry, Japanese Society for Dialysis Therapy. Elevated non-high-density lipoprotein cholesterol (non-HDL-C) predicts atherosclerotic cardiovascular events in hemodialysis patients. *Clin J Am Soc Nephrol*. 2011;6:1112-1120.
5. Liu Y, Coresh J, Eustace JA, et al. Association between cholesterol level and mortality in dialysis patients: role of inflammation and malnutrition. *JAMA*. 2004;291:451-459.
6. Kovesdy CP, Anderson JE, Kalantar-Zadeh K. Inverse association between lipid levels and mortality in men with chronic kidney disease who are not yet on dialysis: effects of case mix and the malnutrition-inflammation-cachexia syndrome. *J Am Soc Nephrol*. 2007;18:304-311.
7. Ritz E, Wanner C. Lipid abnormalities and cardiovascular risk in renal disease. *J Am Soc Nephrol*. 2008;19:1065-1070.
8. Cholesterol Treatment Trialists' (CTT) CollaboratorsKearney PM, Blackwell L, Collins R, et al. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. *Lancet*. 2008;371:117-125.
9. Baigent C, Keech A, Kearney PM, et al., Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366:1267-1278.
10. Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170 000 participants in 26 randomised trials. *Lancet*. 2010;376:1670-1681.
11. Wanner C, Krane V, März W, et al., German Diabetes and Dialysis Study Investigators. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med*. 2005;353:238-248.
12. Fellström BC, Jardine AG, Schmieder RE, et al., AURORA Study Group. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. *N Engl J Med*. 2009;360:1395-1407.
13. Mackay DS, Jones PJ. Plasma non cholesterol sterols: current uses, potential and need for standardization. *Curr Opin Lipidol*. 2012;23:241-247.
14. Costet P. Molecular pathways and agents for lowering LDL-cholesterol in addition to statins. *Pharmacol Ther*. 2010;126:263-278.
15. Silbernagel G, Chapman MJ, Genser B, et al. High intestinal cholesterol absorption is associated with cardiovascular disease and risk alleles in *ABCG8* and *ABO*: evidence from the LURIC and YFS cohorts and from a meta-analysis. *J Am Coll Cardiol*. 2013;62:291-299.
16. Silbernagel G, Fauler G, Renner W, et al. The relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease. *J Lipid Res*. 2009;50:334-341.
17. Silbernagel G, Fauler G, Hoffmann MM, et al. The associations of cholesterol metabolism and plasma plant sterols with all-cause and cardiovascular mortality. *J Lipid Res*. 2010;51:2384-2393.

18. Silbernagel G, Lütjohann D, Machann J, et al. Cholesterol synthesis is associated with hepatic lipid content and dependent on fructose/glucose intake in healthy humans. *Exp Diabetes Res.* 2012;2012:361863.

19. Gylling H, Tuominen JA, Koivisto VA, Miettinen TA. Cholesterol metabolism in type 1 diabetes. *Diabetes.* 2004;53:2217-2222.

20. Miettinen TA, Tilvis RS, Kesäniemi YA. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am J Epidemiol.* 1990;131:20-31.

21. Miettinen TA, Tilvis RS, Kesäniemi YA. Serum cholestanol and plant sterol levels in relation to cholesterol metabolism in middle-aged men. *Metabolism.* 1989;38:136-140.

22. Matysik S, Klünemann HH, Schmitz G. Gas chromatography-tandem mass spectrometry method for the simultaneous determination of oxysterols, plant sterols, and cholesterol precursors. *Clin Chem.* 2012;58:1557-1564.

23. Silbernagel G, Genser B, Nestel P, März W. Plant sterols and atherosclerosis. *Curr Opin Lipidol.* 2013;24:12-17.

24. Simonen PP, Gylling H, Miettinen TA. The distribution of squalene and non-cholesterol sterols in lipoproteins in type 2 diabetes. *Atherosclerosis.* 2007;19:222-229.

25. Arntzenius AC, Kromhout D, Barth JD, et al. Diet, lipoproteins, and the progression of coronary atherosclerosis. The Leiden Intervention Trial. *N Engl J Med.* 1985;312:805-811.

26. Estruch R, Martínez-González MA, Corella D, et al., PREDIMED Study Investigators. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med.* 2006;145:1-11.

27. Plat J, Mackay D, Baumgartner S, Clifton PM, Gylling H, Jones PJ. Progress and prospective of plant sterol and plant stanol research: report of the Maastricht meeting. *Atherosclerosis.* 2012;225:521-533.

28. Demonty I, Ras RT, van der Knaap HC, et al. Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. *J Nutr.* 2009;139:271-284.

29. Kastelein JJ, Akdim F, Stroes ES, et al., ENHANCE Investigators. Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med.* 2008;358:1431-1443.

30. Miettinen TA, Strandberg TE, Gylling H. Noncholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients: relation to basal serum cholestanol. *Arterioscler Thromb Vasc Biol.* 2000;20:1340-1346.

31. Gylling H, Miettinen TA. Baseline intestinal absorption and synthesis of cholesterol regulate its response to hypolipidaemic treatments in coronary patients. *Atherosclerosis.* 2002;160:477-481.

32. Descamps OS, De Sutter J, Guillaume M, Missault L. Where does the interplay between cholesterol absorption and synthesis in the context of statin and/or ezetimibe treatment stand today? *Atherosclerosis.* 2011;217:308-321.

33. Miettinen TA, Gylling H, Strandberg T, Sarna S. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian simvastatin survival study. Finnish 4S Investigators. *BMJ.* 1998;316:1127-1130.

34. Couture P, Lamarche B. Ezetimibe and bile acid sequestrants: impact on lipoprotein metabolism and beyond. *Curr Opin Lipidol.* 2013;24:227-232.

35. Sudhop T, Lütjohann D, Kodal A, et al. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation.* 2002;106:1943-1948.

36. Knopp RH, Gitter H, Truitt T, et al., Ezetimibe Study Group. Effects of ezetimibe, a new cholesterol absorption inhibitor, on plasma lipids in patients with primary hypercholesterolemia. *Eur Heart J.* 2003;24:729-741.

37. Ballantyne CM, Houri J, Notarbartolo A, et al. Effect of ezetimibe coadministered with atorvastatin in 628 patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. *Circulation.* 2003;107:2409-2415.

38. Lin X, Racette SB, Lefevre M, et al. Combined effects of ezetimibe and phytosterols on cholesterol metabolism: a randomized, controlled feeding study in humans. *Circulation.* 2011;124:596-601.

39. Salen G, von Bergmann K, Lütjohann D, et al., Multicenter Sitosterolemia Study Group. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation.* 2004;109:966-971.

40. Cannon CP, Giugliano RP, Blazing MA, et al., IMPROVE-IT Investigators. Rationale and design of IMPROVE-IT (IMProved Reduction of Outcomes: Vytorin Efficacy International Trial): comparison of ezetimibe/simvastatin versus simvastatin monotherapy on cardiovascular outcomes in patients with acute coronary syndromes. *Am Heart J.* 2008;156:826-832.

41. Out C, Groen AK, Brufau G. Bile acid sequestrants: more than simple resins. *Curr Opin Lipidol.* 2012;23:43-55.

42. Brensike JF, Levy RI, Kelsey SF, et al. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI Type II Coronary Intervention Study. *Circulation.* 1984;69:313-324.

43. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. *JAMA.* 1984;251:351-364.

44. Marangon N, Lindholm B, Stenvinkel P. Nonphosphate-binding effects of sevelamer–are they of clinical relevance? *Semin Dial.* 2008;21:385-389.

45. Chertow GM, Burke SK, Dillon MA, Slatopolsky E. Long-term effects of sevelamer hydrochloride on the calcium x phosphate product and lipid profile of haemodialysis patients. *Nephrol Dial Transplant.* 1999;14:2907-2914.

46. Braun J, Asmus HG, Holzer H, et al. Long-term comparison of a calcium-free phosphate binder and calcium carbonate–phosphorus metabolism and cardiovascular calcification. *Clin Nephrol.* 2004;62:104-115.

47. Block GA, Spiegel DM, Ehrlich J, et al. Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. *Kidney Int.* 2005;68:1815-1824.

48. Block GA, Raggi P, Bellasi A, Kooienga L, Spiegel DM. Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. *Kidney Int.* 2007;71:438-441.

49. Suki WN, Zabaneh R, Cangiano JL, et al. Effects of sevelamer and calcium-based phosphate binders on mortality in hemodialysis patients. *Kidney Int.* 2007;72:1130-1137.

50. Xydakis AM, Guyton JR, Chiou P, Stein JL, Jones PH, Ballantyne CM. Effectiveness and tolerability of ezetimibe add-on therapy to a bile acid resin-based regimen for hypercholesterolemia. *Am J Cardiol.* 2004;94:795-797.

51. Rogacev KS, Pinsdorf T, Weingärtner O, et al. Cholesterol synthesis, cholesterol absorption, and mortality in hemodialysis patients. *Clin J Am Soc Nephrol.* 2012;7:943-948.

52. Fukushima M, Miura S, Mitsutake R, Fukushima T, Fukushima K, Saku K. Cholesterol metabolism in patients with hemodialysis in the presence or absence of coronary artery disease. *Circ J.* 2012;76:1980-1986.

53. Shekelle RB, Shryock AM, Paul O, et al. Diet, serum cholesterol, and death from coronary heart disease. The Western Electric study. *N Engl J Med.* 1981;304:65-70.

54. Estruch R, Ros E, Salas-Salvadó J, et al., PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med.* 2013;368:1279-1290.

55. Slinin Y, Guo H, Gilbertson DT, et al. Prehemodialysis care by dietitians and first-year mortality after initiation of hemodialysis. *Am J Kidney Dis.* 2011;58:583-590.

56. Khoueiry G, Waked A, Goldman M, et al. Dietary intake in hemodialysis patients does not reflect a heart healthy diet. *J Ren Nutr.* 2011;21:438-447.

57. Dempsey ME, Farquhar JW, Smith RE. The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. *Circulation.* 1956;14:77-82.

58. Weingärtner O, Böhm M, Laufs U. Controversial role of plant sterol esters in the management of hypercholesterolaemia. *Eur Heart J.* 2009;30:404-409.

59. Anderson JL, Adams CD, Antman EM, et al., 2011 WRITING GROUP MEMBERS; ACCF/AHA TASK FORCE MEMBERS. 2011 ACCF/AHA focused update incorporated into the ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-elevation myocardial infarction: a report of the American College of Cardiology

Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2011;123:e426-e579.

60. European Association for Cardiovascular Prevention & Rehabilitation, Reiner Z, Catapano AL, De Backer G, et al. ESC/EAS Guidelines for the management of dyslipidaemias: the task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J*. 2011;32:1769-1818.

61. Fransen HP, de Jong N, Wolfs M, et al. Customary use of plant sterol and plant stanol enriched margarine is associated with changes in serum plant sterol and stanol concentrations in humans. *J Nutr*. 2007;137:1301-1306.

62. Genser B, Silbernagel G, De Backer G, et al. Plant sterols and cardiovascular disease: a systematic review and meta-analysis. *Eur Heart J*. 2012;33:444-451.

63. Teupser D, Baber R, Ceglarek U, et al. Genetic regulation of serum phytosterol levels and risk of coronary artery disease. *Circ Cardiovasc Genet*. 2010;3:331-339.

64. IBC 50K CAD Consortium. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. *PLoS Genet*. 2011;7:e1002260.

65. CARDIoGRAMplusC4D ConsortiumDeloukas P, Kanoni S, Willenborg C, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*. 2013;45:25-33.

66. Baigent C, Landray MJ, Reith C, et al., SHARP Investigators. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet*. 2011;377:2181-2192.

67. Sato K, Nakano K, Katsuki S, et al. Dietary cholesterol oxidation products accelerate plaque destabilization and rupture associated with monocyte infiltration/activation via the MCP-1-CCR2 pathway in mouse brachiocephalic arteries: therapeutic effects of ezetimibe. *J Atheroscler Thromb*. 2012;19:986-998.

68. Plat J, Theuwissen E, Husche C, et al. Oxidised plant sterols as well as oxycholesterol increase the proportion of severe atherosclerotic lesions in female LDL receptor+/- mice. *Br J Nutr*.; 2013, http://www.ncbi.nlm.nih.gov/pubmed/23773414; 2013.